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Comparative Genetic Mapping of Cellular *rel* Sequences in Man, Mouse, and the Domestic Cat

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SUMMARY

We used in situ hybridization techniques to assign the human c-*rel* locus to the centromere-proximal portion of the short arm of chromosome 2 (2cent-2p13). We also determined the chromosomal location of c-*rel* sequences in the domestic cat and the laboratory mouse by using a human c-*rel* fragment to screen panels of rodent × cat and hamster × mouse somatic cell hybrid DNAs. The c-*rel* locus apparently maintains similar syntenic relationships with other known genetic markers in the human and cat, but displays different linkage relationships in the mouse.

INTRODUCTION

Rearranged chromosomes are often observed in malignantly transformed cells. In some cases, specific chromosomal aberrations seem to be associated with particular disorders, as is true for the human 9;22 translocation that is found in the majority of all chronic myelogenous leukemias (see [1] for review). Recent molecular studies of cancer-related chromosomal abnormalities suggest that rearrangements of this type may initiate transformation by activating cellular oncogenes that lie within, or near, chromosomal breakpoints (reviewed in [1–4,

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5]). One of our ongoing interests is to determine whether cellular homologs of v-*rel*, the transforming gene of reticuloendotheliosis virus strain T (REV-T), are activated by similar means in human and/or rodent (murine) malignancies. Our initial approach was to determine the chromosomal assignment of cellular *rel* (c-*rel*) sequences in humans. In a previous report [6], we provided evidence from rodent × human somatic cell hybrid mapping studies showing that human *rel* sequences reside on chromosome 2. Additional information from a hybrid cell line bearing a human chromosome 2q translocation allowed us to make a provisional regional assignment to 2p11-pter.

Here, we present a more precise localization of the human c-*rel* homolog that was obtained from in situ hybridization of a human *rel* molecular probe to human metaphase chromosomes. We also asked whether c-*rel*'s syntenic relationships have been conserved during the evolution of two other placental mammals, the cat and mouse, whose karyotypes display varying degrees of linkage homology with that of the human [7, 8]. Using somatic cell hybrid panels that preferentially segregate cat and mouse chromosomes, respectively, we have assigned the *rel* homolog to chromosome A3 in the cat and chromosome 11 in the mouse.

MATERIALS AND METHODS

In Situ Hybridization

The procedures that we used were essentially those of Harper and Saunders [9]. One-half microgram samples of pPHHS *rel*-1 [6] DNA were labeled by nick-translation using tritiated TTP and dCTP. The labeled probe was hybridized for 16–18 hrs to metaphase chromosomes prepared from methotrexate-synchronized [10] human primary lymphocytes derived from three phenotypically normal individuals. The hybridizations were performed at 37°C in a reaction mixture containing 50% recrystallized formamide, 10% dextran sulfate, 2 × SSCP (1 × SSCP = 0.15 M NaCl, 0.015 M Na_3 citrate, 0.02 M sodium phosphate buffer), 17 μg/ml sheared salmon sperm DNA, and 35 ng/ml of the probe DNA at 0.5 × 10^7 cpm/μg. Following hybridization, the slides were washed twice in 50% formamide and 2 × SSC at 39°C–40°C for 10 min each and then dehydrated in ethanol. The slides were then dipped in Kodak NTB radiotrack emulsion, dried, and stored in light-tight boxes. Seven days later, they were developed in Kodak Dektol developer for 2.5 min at 15°C. The slides were then G-banded with Wright's stain and scored under a Zeiss plan 63 high-dry objective.

Derivation and Characterization of Somatic Cell Hybrid Panels for Chromosome Mapping of Murine and Feline Genes

Somatic cell hybrids were derived by PEG-mediated fusion of fresh feline lymphocytes to rodent cells (mouse RAG or Chinese hamster E36) lacking the hypoxanthine guanine phosphoribosyl transferase (HPRT) gene, thus allowing selection in hypoxanthine/aminopterin/thymidine (HAT) medium. A panel of 52 hybrids was selected based on retention of low numbers of feline chromosomes in different combinations [11]. Hybrids were genetically characterized by G-trypsin banding [12, 13], G-11 staining, and enzyme typing of 20–28 isozyme markers previously assigned to feline chromosomes at the same passage from which high molecular weight DNA was extracted. Genomic DNA from 40 of these hybrid lines was digested with restriction enzymes and subjected to Southern analyses [14] as described below. Analogous procedures were employed to prepare and characterize the Chinese hamster × mouse cell lines that segregate mouse

chromosomes [15, 16]. The murine cells used in generating these lines were derived from NFS, Balb/c, and AHe mouse strains.

Filter Hybridization

Twenty to 25 micrograms of high molecular weight DNAs were restricted with the designated enzymes using conditions recommended by the suppliers. After 18–20-hr digestions, samples were loaded onto 1.2% agarose slab gels and subjected to electrophoresis for 16–18 hrs at 50–60 V. The DNAs were transferred to nitrocellulose filters [14] and hybridized with a nick-translated DNA fragment (pPHHS *rel*-1) [6] carrying human c-*rel* sequences. The hybridizations were performed for 16–18 hrs at 42°C in a reaction mixture containing 40% formamide; 5 × SSC; 0.025 M sodium phosphate buffer; 5 × Denhardt's reagent (1 × = 0.02% each of bovine serum albumin, ficoll, and polyvinylpyrrolidone); 10% dextran sulfate; 100 μg/ml sheared salmon sperm DNA carrier; and 2 × 10^6 cpm/ml of the nick-translated probe. Following hybridization, the filters were washed twice in 2 × SSC, 0.1% SDS (sodium dodecylsulfate) at room temperature, and twice at 42°C in 0.4 × SSC, 0.1% SDS. Autoradiography was carried out at −70°C using Kodak XAR-5 X-ray film and two Dupont intensifying screens per film. Bands were usually apparent after a 4-hr exposure.

RESULTS AND DISCUSSION

*Regional Localization of Human c-*rel *Sequences by in Situ Hybridization*

REV-T is an acutely transforming type C retrovirus that causes leukemia in young chickens and turkeys. Its oncogene, termed v-*rel,* is thought to have been derived from a turkey cellular gene named c-*rel* [17]. We have recently isolated and sequenced a human genomic DNA segment that carries two exon-like regions that are highly homologous to a portion of v-*rel* and to turkey c-*rel* exons 4 and 5 [6, 17]. The human *rel* segment, designated pPHHS *rel*-1, was used to analyze DNAs from panels of rodent × human somatic cell hybrids to assign the human *rel* locus to chromosome 2. In this study, in situ hybridization of the c-*rel* clone to normal human metaphase chromosome preparations confirmed the assignment of *rel* to chromosome 2 and further localized the gene to the centromere-proximal region of the short arm (2cent-2p13) (fig. 1*A–C*).

In analyses of 90 metaphase spreads from normal human peripheral blood cells, 38 of 208 grains were observed over chromosome 2, well above the 16–17 grains that would have been expected from a random distribution ($P \ll .01$) (fig. 1*A*). The remaining grains constituted a nonspecific background level of approximately two grains per cell, totaling one to three grains per labeled site. Of the 38 grains on chromosome 2, 15 were localized to the region between the centromere and 2p13 (four grains expected; $P \ll .01$), the greatest number (seven) falling over 2p12 (fig. 1*A–C*). On this basis, we conclude that the human c-*rel* sequences are regionally located on the centromere-proximal portion of HSA 2p. This placement is of potential interest because variant (2;8) chromosome translocations involving the IgK region (2p12) have been observed in about 8% of Burkitt lymphomas [18]. It is not known whether alterations in c-*rel* structure or expression also accompany this type of rearrangement. Also, Yunis and Soreng [19] recently localized one of 51 constitutive fragile sites in the human karyotype to HSA 2p13. Fragile sites appear as gaps, or breakpoints, in metaphase chromosomes which, presumably, define regions in the

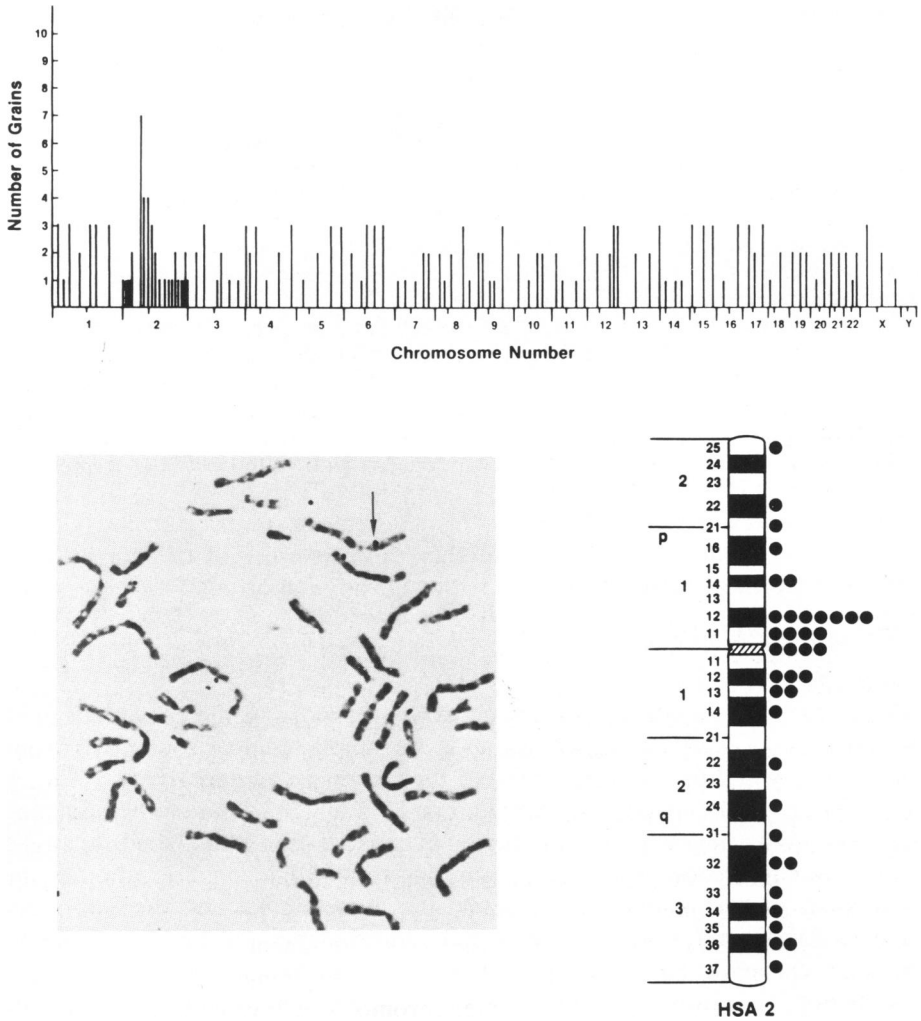

FIG. 1.—*A* (*top*), Histogram of grain distribution compiled from scoring 90 metaphase nuclei hybridized with a human c-*rel* probe. The human chromosome complement is represented schematically on the X-axis, the length of each chromosome being proportional to its percentage of the human karyotype. The short (p) arms lie *to the left* of the short vertical lines, the long (q) arms, *to the right*. Only the region between 2cent and 2p1.13 showed grain counts above random background (three grains per labeled site). *B* (*bottom, left*), Representative metaphase spread showing grain over 2p1.12 (*arrow*). *C* (*bottom, right*), Distribution of grains scored over 38 labeled chromosomes 2. Of these 38 grains, 15 (about 40%) fell over 2cent-2p12. In situ hybridization experiments were performed as described [9, 10].

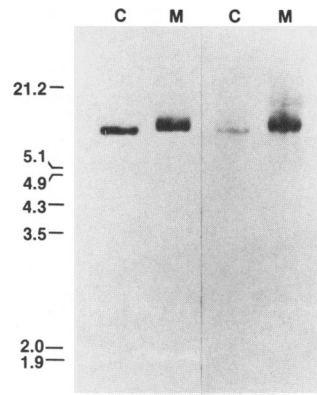

FIG. 2.—Southern transfers [14] of 20-μg aliquots of cat (*C*) and laboratory mouse (*M*) genomic DNAs hybridized with pPHHS *rel*-1 [6] (*lanes 1* and *2*) or with a corresponding subclone of v-*rel* (*lanes 3* and *4*). The cat and mouse DNAs were restricted with *Hin*dIII and *Bam*HI, respectively.

karyotype that are especially prone to mutation and rearrangement. Consequently, we would like to know whether the proximity of the human c-*rel* sequences to the fragile site at 2p13 is biologically important.

Chromosomal Mapping of c-rel *Sequences in the Domestic Cat and Laboratory Mouse*

As is the case for other cellular oncogenes, portions of the c-*rel* locus have been quite conserved during evolution. For example, comparison of two exons of the turkey cellular *rel* gene [17] and their human counterparts [6] shows a 78% identity at the nucleotide level and an 87% match at the predicted amino acid level. We asked whether this degree of conservation also extends to c-*rel*'s genetic linkage relationships by considering the chromosomal location of the c-*rel* locus in two additional mammals, the domestic cat and the laboratory mouse. To this end, we used somatic cell hybrid mapping techniques to determine the chromosomal assignment of c-*rel* in these two species.

In figure 2, we show the results of an initial experiment in which we used the human c-*rel* fragment, pPHHS *rel*-1 (lanes 1 and 2), and a corresponding segment of v-*rel* (lanes 3 and 4), as probes to detect c-*rel* sequences in the genomes of cat and mouse. Identical single bands were observed with each probe, leading us to conclude that the human c-*rel* segment could be used to localize these apparently single copy *rel*-related genes to cat and mouse chromosomes.

Figure 3*A* shows a representative DNA blot in which parental and hamster × cat somatic cell hybrid DNAs [11–13] were restricted with *Hin*dIII and hybridized with the human c-*rel* probe under moderate stringency (40% formamide, 5 × SSC, 42°C).-Using these criteria, we observed one band in the cat genome [7.8 kilobase pairs (kb)] and two hamster DNA fragments (12 and 6 kb). Similar procedures were used to screen a mouse × cat somatic cell hybrid panel in which a single parental mouse *rel* fragment at ca. 18 kb was evident (data not shown). Thus, by digesting hybrid panel DNAs with *Hin*dIII, we could determine which hybrids retained the 7.8-kb feline c-*rel* fragment and, by

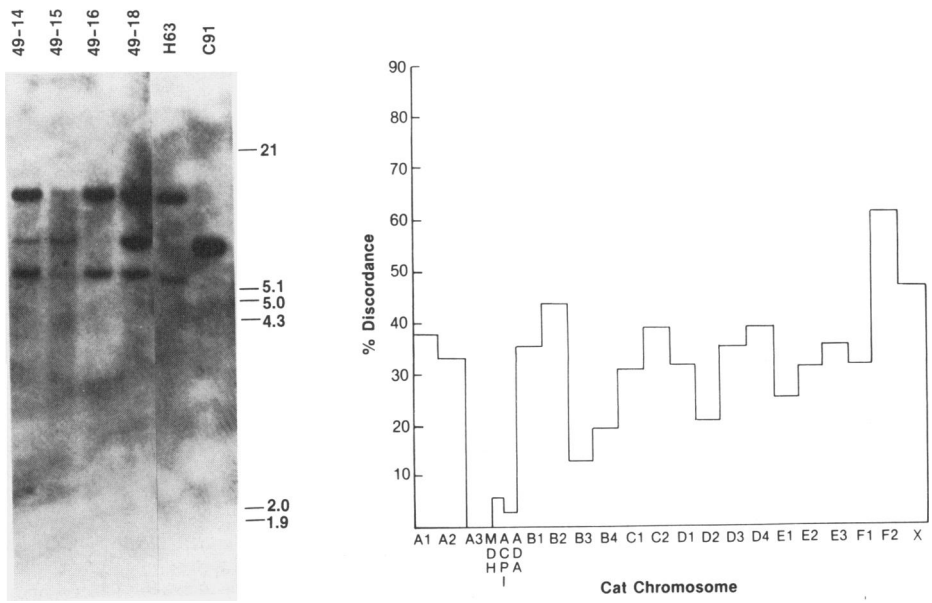

FIG. 3.—*A* (*left*), *Hin*dIII digests of Chinese hamster × cat somatic cell hybrid line (49-14, -15, -16, -18) and parental (H63, C91) DNAs, probed with the human c-*rel* probe. The 7.8-kb cat specific *rel* fragment is present in hybrids 49-14, 49-15, and 49-18, but is absent from 49-16. *B* (*right*), Discordancy analysis of the rodent × cat somatic cell hybrid mapping data. The cat *rel* marker is located on feline chromosome A3, with which it shows no discordancy in the 40 hybrid lines tested.

inference, the chromosome on which it was localized. A discordancy analysis of the feline mapping data (fig. 3*B*) shows that the presence of the *rel*-homologous fragment was 100% concordant with cat chromosome A3 and 95% concordant with three included isozyme loci, malate dehydrogenase (MDH1), acid phosphatase (ACP1), and adenosine deaminase (ADA). Further, the feline c-*rel* fragment was highly discordant with all the other feline chromosomes, permitting assignment of the feline c-*rel* locus to chromosome A3.

The murine homolog of c-*rel* was genetically mapped by a similar analysis of genomic DNAs from a panel of Chinese hamster × mouse somatic cell hybrids that preferentially segregated mouse chromosomes [15, 16]. Figure 4*A* shows a representative Southern transfer experiment in which the DNAs from two hamster × mouse somatic cell hybrid lines were restricted with *Bam*HI and hybridized with pPHHS *rel*-1. The hamster parent exhibited two fragments (ca. 16 and 5 kb), while the mouse parent showed only one (~ 8 kb); this mouse-specific band appeared in only two of the 23 hybrid cell line DNAs that we tested. The results are summarized in the discordancy graph in figure 4*B*, which shows that the mouse 8-kb c-*rel* segment mapped to chromosome 11 with 0% discordancy; all other murine chromosomes were highly discordant for the presence of the c-*rel* restriction fragment. These data permit the assignment of the murine *rel* homolog to mouse chromosome 11, which also bears the genes for two other cellular oncogenes: c-*erb*B [20, 21] and c-*erb*A [20].

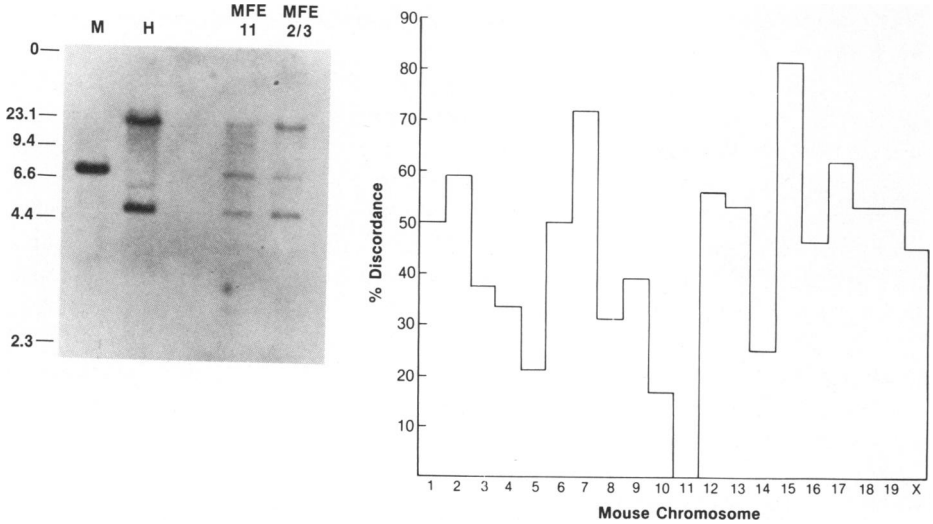

FIG. 4.—*A* (*left*), *Bam*HI digests of two Chinese hamster × mouse somatic cell hybrid line DNAs probed with the human *rel* fragment. The mouse-specific band at approximately 8 kb was present only in these two hybrid DNAs; 21 other hybrid lines were negative. *B* (*right*), Discordancy analysis of the hamster × mouse somatic cell hybrid mapping data.

A comparison of the linkage associations of the *rel* gene and its neighboring loci in the three species is summarized in table 1. Briefly, human chromosome 2 is known to be a recently derived fusion of two acrocentric chromosome arms still separate today in great apes and Old World monkeys. Other than this fusion event, however, the two arms, 2p and 2q, have a conservative evolutionary history (see [22]). In fact, they have been aligned cytologically with their homologs (A3q anc C1p) in the domestic cat [13]. It is not entirely surprising, then, to find that the c-*rel* gene maps to feline A3 considering its position on human 2p. The syntenic chromosome homolog of human 2q is feline chromo-

TABLE 1

COMPARATIVE CHROMOSOME LOCATIONS OF THE c-*rel* GENE AND SYNTENIC MARKER LOCI IN THREE SPECIES

Marker locus	Human chromosome	Cat chromosome	Mouse chromosome
MDH 1.....	2p	A3	ND
ACP 1.....	2p	A3	12
REL	2p	A3	11
IGK.....	2p	ND	6
IDH.....	2q	C1	1
NRAS.....	1p	C1	1
PGD	1p	C1	4
PGM.....	1p	C1	4

NOTE: See references in [7] and [8]. ND = Not determined.

some C1p, which, in addition, has several loci that are located on chromosome 1p in man.

The murine c-*rel* locus, in contrast, does not maintain the linkage associations predicted from its placement in humans and cats. In humans, for example, c-*rel* apparently lies near the IgK locus (at 2p12) while, in mouse, these two markers are found on two different chromosomes (11 and 6, respectively). In this respect, c-*rel* is similar to four other cellular oncogenes (c-*raf*-1, c-*myb*, c-*myc*, and c-*mos*), which also show different syntenic associations in humans and mice [22]. Results such as these have prompted speculation that the mouse karyotype has become shuffled relative to those of humans and cats [22], leaving only short syntenic segments intact between these groups, the lengths of which have been estimated to average about 8.1 centimorgans [23]. The linkage relationships of the unique c-*rel* proto-oncogene in these three index mammalian species is consistent with this hypothesis.

NOTE ADDED IN PROOF: Following submission of our manuscript, Brissenden et al. and Tricoli et al. also published regional localizations of a human transforming growth factor gene to 2p11-p13 and 2p13, respectively (Human Gene Mapping 8; Helsinki Conference, Eighth International Workshop on Human Gene Mapping, 1985. *Cytogenet Cell Genet* 40:1–4, 1985).

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